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Research Article

FORMULATION DEVELOPMENT OF ACYCLOVIR MICROSPHERE USING NOVEL NATURAL POLYMER

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ABSTRACT

Acyclovir [9-(2-hydroxyethoxymethyl) guanine] is an acyclic nucleoside analogue of guanosine that is a potent and selective antiviral agent. It has a relatively short plasma half-life (3 hr). When orally administered, it is slowly and scarcely absorbed from the gastrointestinal tract. The objective of the present work was to formulate and evaluate microspheres of Acyclovir and produced sustained drug delivery. In these 14 batches of acyclovir microspheres was prepared with using natural polymer Kondagogu gum and other ingredients by solvent evaporation technique. The prepared microspheres were evaluated for different parameters i.e % Drug yield, % drug entrapment, shape, surface morphology, particles size, polydispersity index, zeta potential and in-vitro drug release for 48 hrs in phosphate buffer 7.4. The best batch was performed stability studies for 6 months. The research concluded that Acyclovir microspheres could be an alternative for conventional dosage form and other phytochemical in herbs.

Keywords: Acyclovir, Microspheres, Kondagogu gum, Polydispersity index, in-vitro drug release

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INTRODUCTION

Microspheres are one of the multi particulate drug delivery systems and are prepared to obtain prolonged (or) controlled drug delivery, to improve bioavailability or stability and to target drug to specific sites. Microspheres can be defined as solid, approximately spherical particles ranging from 1 to 1000µm, containing dispersed drug in either solution (or) microcrystalline form^{1, 2}. These microspheres are suitable alternatives for conventional dosage forms³. Several methods, including Emulsion solvent evaporation technique⁴, phase-separation or coacervation method⁵, emulsification diffusion method and spray drying method⁶ are commonly used for the preparation of microspheres. The solvent evaporation method has gained much attention due to its ease of fabrication without compromising the activity of drug. This technique offers several advantages and is preferable to other preparation methods such as spray

drying, sonication and homogenization because it requires only mild conditions such as ambient temperature and constant stirring. Thus, a stable emulsion can be formed⁷ and microspheres are formed by the evaporation of an organic solvent from dispersed oil droplets containing both polymer and drug⁸. Microspheres are sometimes referred to as microparticles. Microspheres can be manufactured from various natural and synthetic materials. Glass microspheres, polymer microspheres and ceramic microspheres are commercially available. Solid and hollow microspheres vary widely in density and therefore, are used for different applications. Hollow microspheres are typically used as additives to lower the density of a material. Solid microspheres have numerous applications depending on what material they are constructed of and what size they are. Glass microspheres are primarily used as filler for weight reduction, retro-reflector for highway safety, additive for cosmetics and adhesives, with limited applications in

medical technology. Ceramic microspheres are used primarily as grinding media. Microspheres vary widely in quality, sphericity, uniformity of particle and particle size distribution⁹. Gums are natural polymers, which mainly consists of carbohydrates sometimes with small amounts of proteins and minerals. Increasing demand of natural ingredients over synthetic ones immensely contribute to explore and develop new plant based materials. Gum kondagogu (*Cochlospermum gossypium*) is a tree exudate derived from the Bixaceae family, originating from India. Natural gums are obtained as exudates from different tree species, which exhibit unique and diverse physicochemical properties and have a wide variety of applications¹⁰. Commercially important tree gums include gum arabic, gum karaya, and gum tragacanth¹¹. Karaya polysaccharide (*Sterculia urens*) and gum kondagogu (*C. gossypium*) are used as food additives^{12, 13}. The physicochemical properties and toxicological evaluation of gum kondagogu has been established earlier^{14, 15}. Morphological and structural characterization and physicochemical aspects of gum kondagogu have been elucidated recently, suggesting that this gum belongs to the group of substituted rhamnogalacturonans¹⁶. Understanding of the rheological properties of gum is essential for their application and use as food thickeners, stabilizers, and emulsifiers. Acyclovir (ACV) is known by its IUPAC name as 2-Amino-1,9-dihydro-9-[(2-hydroxyethoxy)methyl]-6H-purin-6-one¹⁷. Acyclovir is a synthetic purine nucleoside analogue with *in vitro* and *in vivo* inhibitory activity against HSV-1, HSV-2 and Varicella Zoster Virus (VZV). Acyclovir is highly selective due to its affinity for the enzyme thymidine kinase (TK) encoded by HSV and VZV. This viral enzyme converts acyclovir into acyclovir monophosphate, a nucleoside analogue. The monophosphate is further converted into tri phosphate by a number of cellular enzymes. Under *in vitro* conditions, acyclovir tri phosphate stops replication of herpes virus DNA. This is accomplished in three ways: competitive inhibition of viral DNA polymerases, incorporation into and termination of the viral DNA chain and inactivation of the viral DNA polymerases. The greater antiviral activity of acyclovir against HSV compared to VZV is due to its enhanced phosphorylation by the viral TK¹⁸. The main objective of this work was to investigate the possibility of obtaining a sustained release formulation of acyclovir microspheres by the solvent evaporation method using gum kondagogu and Investigation of the effect of drug to polymer ratio.

MATERIAL AND METHOD

Material

Acyclovir was procured from Scan Research Laboratories, Bhopal (MP). Kondagogu Gum was purchased from Himedia, Mumbai. Dialysis membrane (MWCO, 15 KDa), Span 80, Tween 80, Glutaraldehyde, Toluene were purchased from Himedia (Mumbai, India). All other reagents and chemicals used were of analytical grade.

Method

Preparation of kondagogu gum microsphere

The microspheres of the polysaccharide, Kondagogu Gum were prepared by emulsifying method using liquid paraffin as a dispersing medium and glutaraldehyde used as a cross-linking agent. Kondagogu gum dispersion (2.5 % w/v) was prepared by mixing of kondagogu gum in double distilled water with Tween 80 (0.5% w/w). Drug was previously dissolved in double distilled water. The prepared, 10 ml of kondagogu gum solution with drug was added drop wise in a beaker containing 100 ml of liquid paraffin light and heavy in ratio of 50:50. Span 80 (1.0% w/v) was previously added in liquid paraffin. The system was kept under stirring at 3000-4000 rpm using two blade mechanical stirrers. 1.5 ml of toluene saturated glutaraldehyde was added to above solution after 30 min of stirring. Stirring was continued for 4 hr at 40°C at 4000 rpm. The microspheres were separated from dispersion medium by centrifugation after stirring and washed two times with petroleum to remove liquid paraffin and then washed three times with acetone. Dispersion was poured in petridish to remove acetone. After complete evaporation of acetone, dried drug loaded microsphere were collected and stored in tight container for further evaluation.

Optimization of microsphere

Optimization of microsphere formulation was carried out by optimizing the different dependent and independent process and formulation variables. Optimization was carried out on the basis of particles size, polydispersity index and % drug entrapment and it is done by changing the one variable and kept constant for other variables given in table 1. The temperature was maintained at 40°C and the concentration of toluene, saturated glutaraldehyde was used 1.5 ml in the preparation of each formulation.

In Vitro Characterization of Microspheres

Particle size, polydispersity index

Average particles size, polydispersity index (PDI) of prepared microsphere was determined using zetasizer (DTS were.4.10, Horriba instrument, India). The microsphere formulation was diluted with deionized water (1:9 v/v) and analysed for average size and PDI and it was performed at the department of pharmaceutical science, VNS pharmacy college Bhopal, India Table 1.

Shape and surface morphology

The shape and surface morphology of the microspheres were investigated using scanning electron microscopy (IISER, Bhopal). The microspheres were fixed on supports with carbon-glue, and coated with gold using a gold sputter module in a high-vacuum evaporator. Samples were then observed with the Scanning Electron Microscope at 10 kV.

Determination of drug content

The amount of drug entrapped in the microspheres was determined using a UV spectrophotometer. The weighed amount of the microspheres was incubated with PBS, pH 7.4, for 48 h. It was centrifuged at 10,000 g for 30 min and the supernatant was diluted 10 times before

analysis into the UV spectrophotometer system at λ_{max} 252 nm.

***In Vitro* drug release from microspheres**

The drug release was performed in PBS (7.4 pH) for acyclovir loaded kondagogu gum microsphere. The drug release was performed in PBS (7.4 pH) for prepared microsphere using dialysis bag technique. In this study suspension of microsphere equivalent to 20 mg of drug was taken in dialysis tubing (MWCO, 15KDa, himedia) and placed in a beaker containing 50ml of PBS pH 7.4. The dialysis bag retains microsphere and allows passing of free drug into the dissolution media. Temperature was maintained at $37 \pm 1^\circ\text{C}$ throughout the study. The samples were withdrawn after specified time intervals that is 0.5, 1, 2, 3, 4, 5, 6, 7.8.12, 24 and 48 hrs and replaced with the same volume of fresh PBS pH 7.4 and analyzed for drug concentration by using UV spectrophotometer a λ_{max} 252 nm Table 2.

RESULT AND DISCUSSION

The mean diameter of glutaraldehyde cross linked microspheres of kondagogu gum increased from $56.70 \pm 2.15 \mu\text{m}$ to $88.15 \pm 4.25 \mu\text{m}$ with increasing

polymer concentration from 1.0 to 3.0 % w/v. In the present investigation a 2.5% w/v kondagogu gum concentration was found to be optimized which give the required size of microspheres. The average particle size of microspheres increased with increasing polymer concentration, since at higher concentrations the polymer solution dispersed into larger droplets due to increasing the viscosity of polymer solution and it was the reason behind the enhancement of average particle size of microsphere. Mean particle size and size distribution were studied to observe the effect of drug concentration. It was found from previous study that there was no major change observed on particle size and size distribution of microsphere with varying concentration of the cross linking agent. Percent encapsulation efficiency has increased up to $77.37 \pm 3.15\%$ with increasing polymer drug concentration from 15% to 25 % w/w. But further increasing the concentration of drug, there was no significant enhancement was found in entrapment efficiency. The in vitro dissolution profile of acyclovir in PBS pH 7.4 was found 83.46% after 48 hr for optimized formulation (KMTSD-13) and follow the matrix diffusion Higuchi release kinetics Fig 1-6.

Table 1: Formulation of microsphere

F. Code	Kondagogu Gum (%w/v)	Tween-80 (%)	Span-80 (%)	Stirring Speed	Drug Conc. (% w/w)	PDI	Particle Size (μm)	% Drug Entrapment
KMS-1	1.5	1	0.5	2000		0.210 \pm 0.005	56.70 \pm 2.15	-
KMS-2	2	1	0.5	2000		0.316 \pm 0.025	64.34 \pm 3.26	-
KMS-3	2.5	1	0.5	2000		0.153 \pm 0.023	71.62 \pm 4.56	-
KMS-4	3	1	0.5	2000	-	0.331 \pm 0.045	88.15 \pm 4.25	-
KMT-5	2.5	1	0.5	2000	-	0.263 \pm 0.012	65.45 \pm 0.12	-
KMT-6	2.5	1.5	0.5	2000	-	0.143 \pm 0.045	58.56 \pm 0.45	-
KMT-7	2.5	2	0.5	2000	-	0.201 \pm 0.036	47.36 \pm 0.32	-
KMTS-8	2.5	1.5	0.75	2000	-	0.150 \pm 0.012	38.62 \pm 3.26	-
KMTS-9	2.5	1.5	1.0	2000	-	0.147 \pm 0.054	32.54 \pm 4.56	-
KMTS-10	2.5	1.5	1.25	2000	-	0.164 \pm 0.005	28.27 \pm 2.66	-
KMTS-11	2.5	1.5	1.0	2000	-	0.170 \pm 0.012	31.63 \pm 4.53	-
KMTS-12	2.5	1.5	1.0	3000	-	0.135 \pm 0.023	22.54 \pm 3.12	-
KMTS-13	2.5	1.5	1.0	4000	-	0.120 \pm 0.015	18.37 \pm 2.12	-
KMTSD-11	2.5	1.5	1.0	4000	15	0.125 \pm 0.012	16.63 \pm 4.56	68.63 \pm 4.56
KMTSD-12	2.5	1.5	1.0	4000	20	0.132 \pm 0.023	20.54 \pm 3.15	74.54 \pm 3.12
KMTSD-13	2.5	1.5	1.0	4000	25	0.115 \pm 0.015	23.37 \pm 2.16	77.37 \pm 3.15
KMTSD-14	2.5	1.5	1.0	4000	30	0.280 \pm 0.015	23.37 \pm 2.12	77.62 \pm 2.02

Table 2: In vitro drug release

S. No.	Time interval (h)	Plain drug	Acyclovir Loaded Microsphere
1	0.5	36.59	08.43
2	1	49.15	16.53
3	2	72.79	28.26
4	3	91.38	35.68
5	4	98.49	46.35
6	5		54.23
7	6		62.45
8	8		69.38
9	12		74.43
10	24		79.34
11	48		83.46

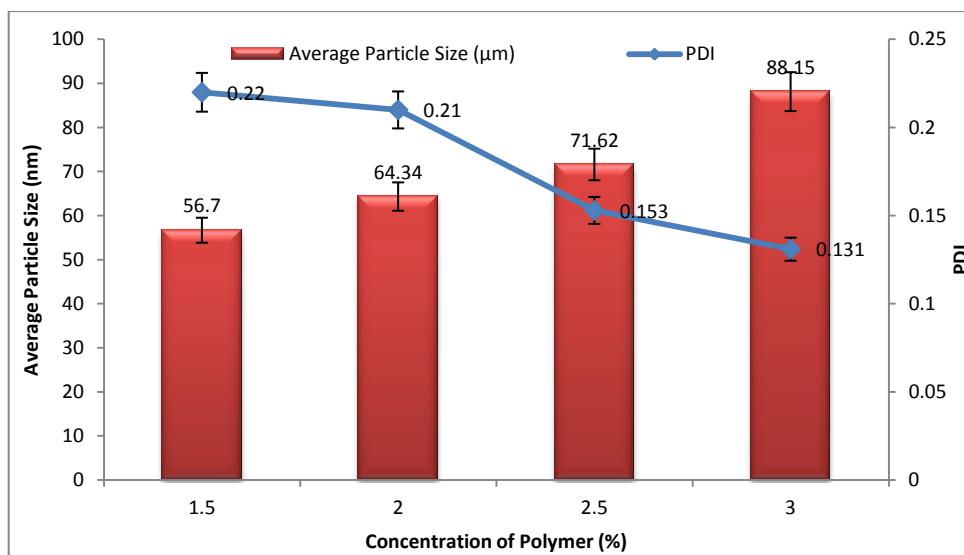


Figure 1: Effect of Kondagogu gum concentration on average particle size and PDI of microsphere

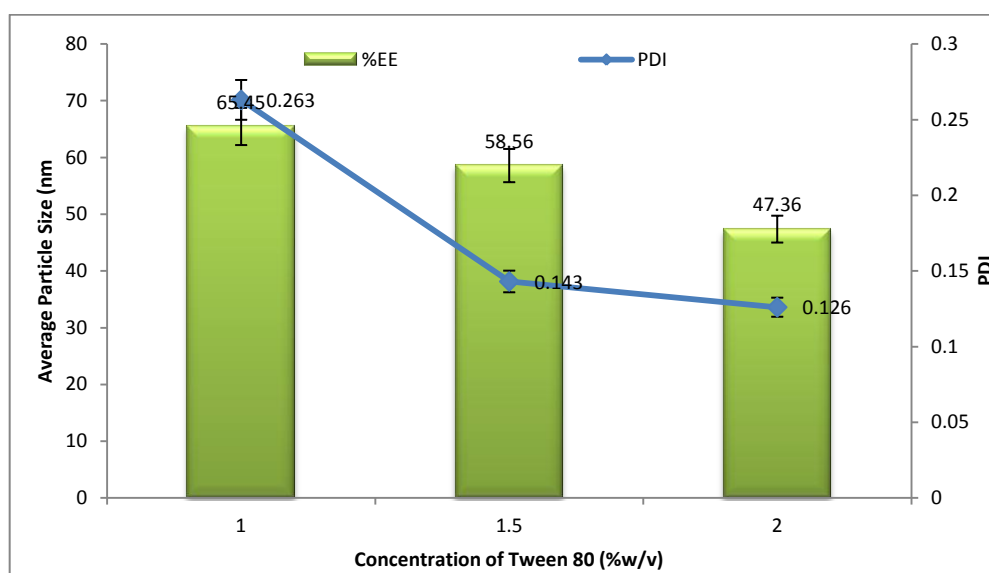


Figure 2: Effect of concentration of Tween 80 on average particle size and PDI of microsphere

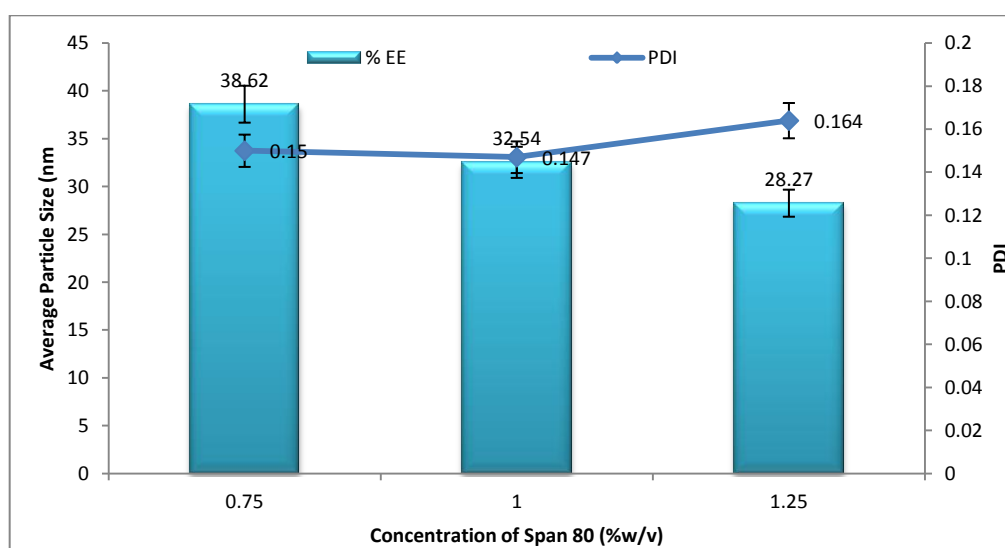


Figure 3: Effect of Span 80 concentration on average particle size and PDI of microsphere

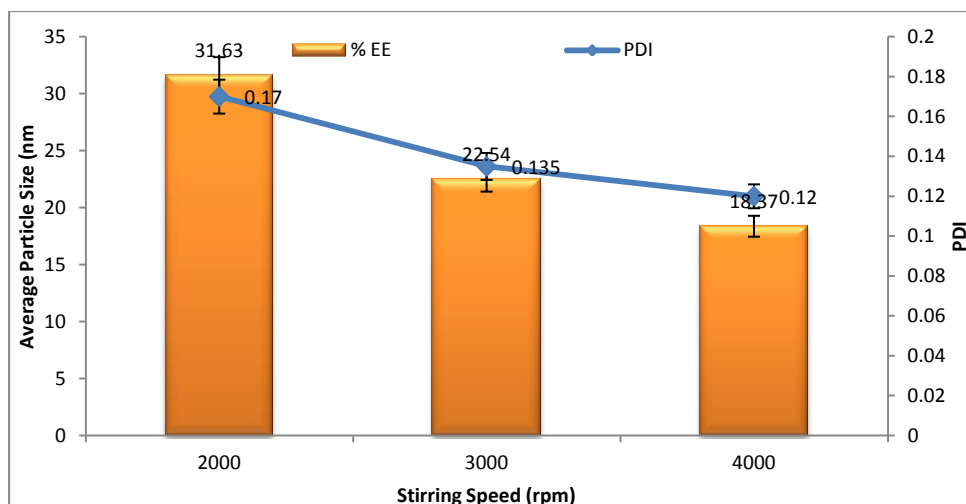


Figure 4: Effect of stirring speed concentration on average particle size and PDI of microsphere

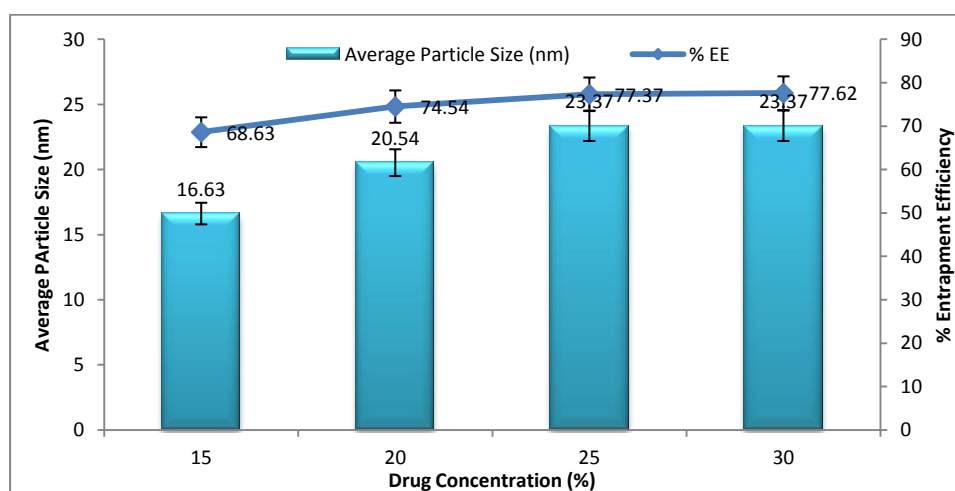


Figure 5: Effect of drug concentration on average particle size and % entrapment efficiency of microsphere

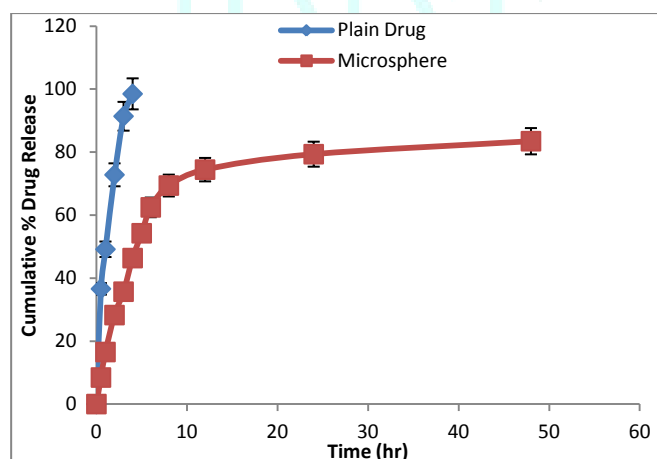


Figure 6: Cumulative % acyclovir release

CONCLUSION

It was concluded that from this study that the microsphere can be prepared from kondagogu gum by emulsifying solvent evaporation method and can be loaded with drug acyclovir for its sustained delivery in GIT system. The prepared microspheres were optimized

for different formulation and process variables concentration and found that microsphere was uniform and acceptable size range. They were found smooth and spherical in shape. The optimized formulation was found significant loading efficiency of acyclovir that can release the acyclovir in sustained manner which was followed matrix diffusion Higuchi release kinetic.

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